(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 December 2002 (05.12.2002)

PCT

(10) International Publication Number
WO 02/096384 A1

(51) International Patent Classification[7]: A61K 7/48

(21) International Application Number: PCT/KR02/00997

(22) International Filing Date: 27 May 2002 (27.05.2002)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
2001/29430 28 May 2001 (28.05.2001) KR

(71) Applicant *(for all designated States except US)*: **BIOLAND LTD.** [KR/KR]; 39-4 Songjeong-ri, Byungchun-myun, Chonan-si, 330-863 Chungchongnam-do (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LEE, Kun-Kook** [KR/KR]; 102-1207, Imkwang APT, Yongam-dong, Sangdang-gu, Chongju-si, 360-181 Chungchongbuk-do (KR). **CHOI, Jung-Do** [KR/KR]; 106-101, Samjeongbackjo APT, 310, Singbong-dong, Heungduk-gu, Chongju-si, 361-111 Chungchongbuk-do (KR). **KIM, Ki-Ho** [KR/KR]; 207-1903, Sungjisaimal APT, 874, Sinbang-dong, Chonan-si, 330-260 Chungchongnam-do (KR). **KO, Kang-Il** [KR/KR]; 101-601, Jungang APT, Sangdong-ri, Book-myun, Chonan-si, 330-853 Chungchongnam-do (KR). **LEE, Jae-Soeb** [KR/KR]; 101-408, Sinhan APT, Gajeon-ri, Byungchun-myun, Chonan-si, 330-861 Chungchongnam-do (KR). **HAN, Chang-Sung** [KR/KR]; 1001, Sinsegae APT, Byungchun7-ri, Byungchun-myun, Chonan-si, 330-861 Chungchongnam-do (KR).

(74) Agent: **YOON, Eui-Seoup**; Yoones & Co, 3F Namdo Bldg., 823-24 Yoksam-dong, Kangnam-gu, 135-080 Seoul (KR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/096384 A1

(54) Title: ANTI-AGING COSMETICS COMPOSITION CONTAINING PHENOLIC COMPOUND

(57) Abstract: The purpose of the present invention relates to the composition of cosmetic materials containing phenolic components obtained from the seed of Areca Catechu Linn., which has excellent anti-aging effects including elastase inhibitory effect, anti oxidation effect, free radical scavenging effect, hyaluronidase inhibitory effect, tyrosinase inhibitory effect, and so one. In the present invention, the phenolic components are produced through the following processes. After the seed of Areca Catechu Linn. Is dried, anhydrous or aqueous alcohol is added into the said dried seed of Areca Catechu Linn., from which the extract is drawn. And then, the extract is partitioned in the solution which is composed of 50 % water and 50 % organic solvent such as aceton, ethylacetate, diethylether, benzene, chloroform, hexane, and butanol. Finally, the phenolic components are obtained by using silicagel column chromatography, TLC and HPLC. from the organic layer of the solution.

ANTI-AGING COSMETICS COMPOSITION CONTAINING PHENOLIC COMPOUND

TECHNICAL FIELD

This invention is related to anti-aging cosmetic composition containing phenolic compound extracted from the seed of Areca Catechu L.

BACKGROUND ART

The aging process of the life applies to human body. So is our skin. Skin is very important in health control. For that reason, researches that determine the aging mechanism of the life and its process of related enzymes are extremely important. The aging process is taking place continuously in metabolism, and even gets rapid process in state of disease or stress. Particularly, skin is exposed to ultraviolet rays that make continuous chemical reaction. In addition, smoking, pollution, and virus infection became major cause of skin aging. The active oxygen plays a leading role for aging of skin cell and organism. These active oxygen cause major damages on organism by destroying antioxidant defense system constructed with antioxidant enzymes and non-enzymatic antioxidant. These active oxygen cause per-oxidation of the lipid, activation of the protease that destroys cells, chain cleavage and abnormal cross linkage of elastic fibers which are collagen and elastin, cleavage of hyaluronic acid chain, accelerating reaction of melanin formation and DNA oxidation. As a result, the skin aging process such as elastic reduction, increase of wrinkles and freckles formation will be accelerated (J. Soc. Cosmet. Sci. Kore., 23, pp75-132, 1997). Therefore, we need the system that can restrain to delay and inhibit the skin aging not just inside the body but also on the skin.

Skin is one of the most complex parts of human body. It is divided into three parts; epidermis, corium, and subcutaneous and many accessory

organ such as sebaceous gland, eccrine gland etc. Photoaging by UVA and natural aging seriously damages such dermal connective tissue of skin. When rays are applied to skin, either it will be dispersed, reflected, or absorbed by skin. Those absorbed rays transfers light energies to hexane, amino acid, melanin, and various chromophores. As a result, chromophores become excited state or produce free radical by photolysis. In addition, rays can produce super oxide anion radical by interaction with free oxygen molecules existing in internal body. These super oxide anion radicals take steps of chain reaction which produce noxious active oxygen such as singlet, oxygen ($^1O_2$), hydroxyl radical (OH), or H_2O_2 (Pharmaceutical magazine (daily), p111, pp103-109, 1991). Super oxide radical produce various noxious active oxygen during the reaction of monocyte, neutrophil, mcarophage or mast cell with external substance or immune complexes (PNAS USA p77, pp115-1163, 1980). This noxious active oxygen reacts with hexane, lipid, protein, and carbohydrate, and cause direct damages to organism, and produce per-oxy radical or peroxide causing broaden damages on organism by its chain reaction. In addition, absorbed UV activates phospholipase A by its photons. This phospholipase A leads the release of the arachidonic acid, which is unsaturated fatty acid, ester bonded to the 2nd carbon of phosphatidylcholine of phospholipid forming cell membrane (Dermatol., p9, pp11-15, 1990). The arachidonic acid, which is eicosanoid comprised of 20 carbons, by action of cyclooxygenase and lypoxygenase, produces prostaglandins and leukotrienes which are become inflammation factor. This prostaglandin activates hyaluronidase in mast cell, and secretion of histamine and chemical material cause inflammation such as vasodilatation and protein damages. In addition, by stimulus of UV, tissue and cells get damages by action of protease such as elastase released from polymorphonuclear leukovytes, and inflammation can be amplified by excess noxious oxygen produced during the phagocytosis process. The

elastic fiber forms bridge bond with collagen fiber at epiderm of skin. The elasticity of the skin declines by the action of the elastase and sagging is formed as human body gets old. In systematic view, increasing permeation of inflammation, deficiency and cohesion of elastin fiber, and decrease of collagen fiber can be observed. And in biochemical view, great increase of activity of elastase can be observed. The elastase is known as only enzyme that can decompose elastin, and the inhibition of this elastase can retard skin aging fundamentally. Prior methods for inhibition of skin aging are mixing of nutrient, additive, refreshing agent, anti-inflammation agent, or elastin into cosmetic compound, and apply it to skin organism which bridged bond has damaged. But in fact, these methods have limits in inhibition of aging. Therefore, fundamental inhibition of elastin and collagen decomposing for skin elasticity is needed. Today, many researchers are trying to find a new functional compound because prior methods for inhibition of skin aging have limits in its function. Particularly, synthetic peptide derivative, which has similar structure with existing animal extract or collagen and elastin, is being used today, but its safety and stability is quite regrettable. On the other hand, various natural substances, used for herb medicine, have great stability and contain various useful medicine ingredients. For that reason, many researchers are looking for more of these natural substances to work with.

On the following of described standpoint, this invention, focusing on searching of fundamental substances that are effective in keeping elasticity of skin, have searched new substances among natural plans from various places. As a result, inventors have founded that the extract from areca catechu is an excellent substance of elastase inhibition and shows great effects in inhibition of skin aging and keeping elasticity of skin (Republic of Korea Patent Application: 1997-78817, 1999-0056924; Int. J. Cosmet. Sci., p21, pp71-82, pp275-295, 1999).

These inventors have extracted area catechu with 90% ethanol, and have performed a fraction with various solvents, and finally have measured the elastase inhibition activeness to find an active ingredient inhibiting excess secretion of elastase from nutrophil in epiderm of skin. For noticeable fraction, inventors have applied silica gel column chromatography, preparative TLC, and reverse HPLC. The active ingredient in reverse HPLC was confirmed that it is a phenolic compound with its various color reaction, UV, and IR spectrum.

DISCLOUSEURE OF THE INVENTION

Therefore, the purpose of this invention is to offer a phenolic compound extracted from areca catechu L.

The purpose of this invention is to make an excellent cosmetic composition that contains phenolic compound and has great-integrated effect in inhibition of skin aging such as elastase inhibition, antioxidant effect, free radical elimination, hyaluronidase inhibition, and tyrosinase inhibition.

On the other hand, this invention is focused on offering of reasonable method in extracting phenolic compound that has elastase inhibition effect from areca catechu L.

Areca catechu L. is widely distributed in south China, Taiwan, and Malaysia, and cultivated in various tropical regions. Areca catechu L. can be used as peptic, digestive, anthelmintics and known as very effective in treatment of indigestion, constipation, stomachache, and vermicide (General comment 5-20037).

The purpose of this invention can be achieved by phenolic compound prepared by steps of extracting ingredients from dried seed of Areca catechu L., with anhydrous or aqueous alcohol, or one or more organic solvent selected from the group comprised of acetone, ethyl acetate, diethyl ether, benzene, chloroform, hexane and butanol; and obtaining phenolic

compound by silica gel column chromatography, preparative TLC, and reverse HPLC from said extract. The purpose of this invention can be achieved by creating cosmetic composition that contains phenolic substances extracted with above method.

This invention offers synthetic cosmetic composition that contains both of whitening and wrinkle elimination cosmetic substances. These substances contain phenolic compound purified from Areca catechu L. extract (Republic of Korea Patent Application: 1997-78817, 1999-0056924; Int. J. Cosmet. Sci., p21, pp71-82, pp275-295, 1999) and it is very effective in total inhibition of skin aging.

To gain proper effects matching with purpose of this invention, cosmetic composition should contain 0.00001~5.0 weight %, preferably, 0.001~1.0 weight % of phenolic compound of the present invention.

This cosmetic compound has no limits in its form of products. For example of the wrinkle elimination and whitening cosmetics, moisture facial lotion, nutrition facial skin, eye cream, nutrition cream, massage cream, cleansing cream, cleansing water, essence, powder, or pack are available.

In addition, for each cosmetic compound product, manufacturers can mix other selected substances such as additional wrinkle elimination and whitening substances into phenolic compounds extracted from Areca catechu L.

BEST MODE FOR CARRYING OUT THE INVENTION

Hereinafter, the preparations of cosmetic compositions according to the present invention, and experiments for proving the effects are described in detail by referring to the examples and experimental examples. However, it is apparent that the present invention is not limited to these examples.

For the experimental examples, inventors isolated and purified phenolic compounds extracted from areca catechu L. and have prepared

composition containing phenolic substance (operation example) and composition without phenolic substance (comparison example).

Preparation example: isolating and purifying method for phenolic compound extracted from Areca catechu L.

Pits of Areca catechu L was dried and pulverized after cleaning with pure water. 1-15 times low alcohol such as ethanol, methanol, propanol, and butanol; or hexane, acetone, ethyl acetate, diethy lether benzene, or chloroform as solvent was applied to pulverized extract. Mixture was heated for 4-20 hours at 50-95°C under the condition of prohibiting the evaporation of the active ingredient by cooling condenser. Alternatively, Mixture was impregnated with solvent for -15 days. This areca extract was passed through evaporator with cooling condenser and evaporated solvent was recovered. After concentrating extract under the reduced pressure, dried areca extract was obtained.

100g of 90% ethanol extract was dissolved into distilled water and the same amount of hexane was added. After separating the hexane layer and concentrating it under reduced pressure, 3.39g of hexane layer extract was obtained. After adding same amount of chloroform into water layer, chloroform layer was separated and concentrated under reduced pressure , and 1.05g of chloroform layer extract was obtained. After adding same amount of ethyl acetate into water layer and ethyl acetate layer was separated and concentrated under reduced pressure , and 5.97g of powder was obtained. After adding butanol into water layer and separating layers , 21.90g of butanol layer and 30.29g of water layer were obtained. Inhibition activities for elastase were measured for each extract.

100g of silica gel (70-230 mesh) was added into ethyl acetate and charged in column. 1.8 g of butanol layer powder obtained from fraction was dissoved into methanol and loaded, and eluted with 600ml of ethly

acetate, 600ml of methanol and 600ml of water. After concentrating each fraction under reduced pressure, inhibition activities of elastase were measured in 100㎍/㎖ concentration.

TLC was applied in Each step and ethlyacetate : methanol : water = 9 : 2: 2 were used as development solvent. For coupling, TLC panel was heated after spreading anisealdehyde reagent on it. Preparative TLC was performed under the condition of ethtlyacetate : methanol : water = 9 : 2 : 2 for high active fragment from silica column chromatography elution and active part was separated. This active part was filtered after dissolving into methanol, and concentrated under reduced pressure.

For purifying inhibition substance of enzyme activity, HPLC was performed for the sample from preparative TLC. YMC-pack ODS-AQ column (10x250mm) and methanol : water = 30 : 70 (flow rate: 1.5㎖/min) were used. The result was detected at UV 280 nm. The phenolic compound was identified after those each confirmed peak was separated, and the anti-aging effect such as elastase inhibition activities was measured.

[Table I] Example and Comparison example

	Weight (%)	Weight (%)
Compound	Example	Comparison example
Phenolic compound	0.005	-
Butylene glycol	2.0	2.0
Propylene glycol	6.0	6.0
Carboxy vinyl polymer	0.5	0.5
Bees wax	2.0	2.0
Vaseline	7.0	7.0
Polyoxyethylene	2.0	2.0

Sorbitan Monostearate		
Sorbitan sesquioleate	2.5	2.5
Squalene	3.0	3.0
Liquid paraffin	10.0	10.0
Triethanolamine	0.5	0.5
Tocopheryl acetate	0.1	0.1
Aroma, Antiseptic	0.3	0.3
Purified water	To 100	To 100

[Experimental Example. I]

[Identification test for the Ingredient which inhibit enzyme activity]

(i) IR Spectrum: Inhibition substance from HPLC separation was processed into KBr pellet and measured with Jasco FT/IR-300E

(ii) UV Absorb Spectrum: Inhibition substance from HPLC separation was dissolved into methanol and optical absorptionwas measured from 200nm to 800nm using UV/Vis spectrophotometer.

(1) Amino Acid: ninhydrin reagent (0.2% aqueous solution) was added to extracted liquid. It was observed if its color changes to purple when it is boiled in water.

(2) Phenolic compound, flavonoid, tannin
: 2.5% $FeCl_3$ solution in ethanol was dropped to extracted liquid. It was observed if its color changes to dark green or if something is deposited.

(3) Saccharide : 2-3 drops of 5% α –naphthol alcohol reagent were added to extracted liquid. It was observed the reddish purple color was appeared in surface when sulfuric acid was applied.

(4) Triterpenoid, Saponin : Anhydride acetate was dropped to extracted liquid and mixed with sulfuric acid. It was observed if its margin color

changes to reddish brown.

[Experimental Example 2]

[Test for the free radical elimination effects]

Free radical elimination function was measured in purified active ingredients from areca catechu. 100㎕ of 0.3 mM of 1, 1-diphenyl-2-pycrylhydrazyl radical (DPPH) solution in methanol was added into 100㎕ of sample solution in methanol, and reaction was processed for 30 min at 37°C, and its optical absorption was measured at 517 nm. For the comparison example, methanol was used instead of sample of the present invention

[Experimental Example 3]

[Measurement for the Hyaluronidase inhibition activity]

Hyaluronidase inhibition effects were measured for purified and extracted substance from areca catechu. Concentrated sample was dissolved into methanol. 50㎕ of hyaluronidase solution (hyaluronidase type IV-S, 10100 units/ml) was added to 100㎕ of sample solution and it was kept for 20 min at 37°C. Next, 100㎕ of enzyme activation solution (2㎎ compound 48/80/15㎎ $CaCl_2 \cdot H_2O$/4㎖ acetate buffer solution) was added to sample solution and it was kept for 20 min at 37°C. Next, 250㎕ of hyaluronidase solution (1.2㎎/0.1M acetate buffer solution) was added to sample solution and mixture was stirred for 40 min at 37°C in water bath. Next, 0.4 N NaOH(100㎕) and 0.4 M boron calcium solution(100㎕) was added. Mixture was stirred and boiled in water about 3 min at 95°C, and cooled. Next, 2㎖ of p-dimethylaminobenzaldehyde (p-DMAB) solution (5g/acetic acid 44㎖, 10N HCl 6㎖, dilute 10 times with acetic acid before any use) was added and it was kept for 20 min at 37°C in water bath. Optical absorption was measured at 585 nm for hyaluronidase inhibition

effect and inhibition activity was calculated by the following method.

$$\text{Hyaluronidase inhibition percentage (\%)} = \frac{\text{Optical density(OA) of comparison} - \text{OA of sample}}{\text{Optical absorption of Comparison}} \times 100$$

In Blank test, purified sample and p-DMAB solution are added, and methanol was used instead of sample solution for comparison

[Experimental Example 4]

[Measurement for the tyrosinase inhibition activity]

Tyrosinase separated and purified from mushroom was bought from SIGMA and used. Tyrosin, which is substrate, was dissolved into 0.05M of sodium phosphate buffer solution (pH 6.8) to make 0.1㎎/㎖ solution for this test.

Phenolic compound was diluted by buffer solution and dissolved in proper concentration to use as sample solution. O.5㎖ of tyrosin solution was dropped into test tube with 0.5㎖ of sample solution, and this test tube was kept for 10 min at 37°C. Next, 0.5㎖ of 200U/㎖ tyrosinase was added to test tube and left to act on for 10 min. 0.5㎖ of buffer solution was added for comparison. This test tube containing reaction fluid was put on ice for rapid cooling to stop reaction, and optical absorption was measured at 475nm using spectrophotometer. Following method is for computing increased tyrosinase activation effect.

Tyrosinase activation

Inhibition

$$\text{percentage (\%)} = 100 - \left[\frac{\text{Optical absorption of sample}}{\text{Optical absorption of comparison}} \times 100 \right]$$

Following tables 2 and 3 show yield and inhibition effects of each separated purifying steps, and table 4 shows identification test result.

[Table2]

Yield and elastase inhibition activity of areca extract which is extracted by organic solvent

Solvent fractions	Yield	$IC_{50}(\mu\ell/m\ell)$ Elastase inhibition effects
90% Ethanol	100	64.05
Hexane	3.39	>500
Chloroform	1.05	>500
Ethyl acetate	5.97	>500
Butanol	21.30	104.93
Water	30.29	31.04

- $IC_{50}(\mu\ell/m\ell)$ is the concentration which enzyme inhibition percentage is 50%.

[Table 3] Yield and inhibition effects of each separated purifying steps

Separated purifying steps	Yield (%)	$IC_{50}(\mu\ell/m\ell)$			
		Elastase	Hyaluronidase	Tyrosinase	Free radical elimination
Extract of BuOH layer	100	104.9	416	120	6.9
Silica column Chromatography	28.2	31.6	-	78	5.0

Collecting after TLC	3.9	25.0	-	41	6.5
Phenolic compound After HPLC	2.8	26.9	210	49	6.0
Comparison sub. Vitamin C	-	-	-	-	19.0
Comparison sub. BHT	-	-	-	-	18.5
Comparison sub. Licorice extract	-	-	330	-	-
Comparison sub. Urusolic acid	-	31.0	-	-	-
Comparison sub. Arbutin	-	-	-	65.2	-

The phenolic compound purified from areca shows 26.9㎕/㎖ and 60.8㎕/㎖ of IC_{50} value for porcine pancreas elastase (PPE) and human neutrophil elastase (HNE) orderly. This value is much higher than existing elastase inhibitor, oleanolic acid (76.5㎕/㎖, 219.2㎕/㎖ each) and ursolic acid (31.0 ㎕/㎖ and 118.6㎕/㎖ each), and this phenolic compound shows competitive reaction with substrate to PPE and HNE. Furthermore, in test to determine whether this phenolic compound has an ability to eliminate free radical, it is found that concentration value eliminating 50% of free radical is 6㎕/㎖ and it is much higher than standard compound such as vitamin C (19 ㎕/㎖) or butylated hydroxyl toluene (18.5㎕/㎖). Inhibition process on hyaluronidase, which is activated in mast cell, is very effective as it shows 210㎕/㎖ of IC_{50} value. In addition, IC_{50} value of inhibition process for tynosinase , which is known as whitening effect measurement, is 49㎕/㎖. This value is almost same as existing whitening composite such as kojic acid (58.2㎕/㎖) and arbutin (65.2㎕/㎖).

[Table 4]

Characteristics of phenolic compound purified and extracted from areca catechu.

Appearance	Weakly brown
Melting point	>300 ^{0}C
IR cm^{-1} (KBr)	3367, 1617, 1110
UVmax (H_2O/nm)	279
Color	
Reaction (+)	$FeCl_3$
(-)	Ninhydrin
	p-anisidine-phthalic acid
	Aniline-Diphenylamine
	Molish
	Dragendorff
Solubility	
Soluble	H_2O, methanol
Insoluble	Other common organic solvents

UV, IR spectrum was obtained to find out what kind of compound is the sample, which is purified from areca catechu to HPLC step. And various color reaction was performed.

As a result in UV spectrum, maximum optical absorption was displayed at 280nm and it was able to estimate that this compound contains aromatic ring. In addition, some absorption was shown at visible ray wave and this state can explain why the inhibition substance has brown color (Data not shown). As a result in IR spectrum, it was able to estimate 3367.10 (OH),

1617.02 (C=C conjugated), and 1110.31 (C-0) cm^{-1}.

There is color reaction to confirm the presence of an active ingredient (Research in Natural Substance Chemistry, Publishing Department of Seoul National University). It is needed to find out if, out of result in UV spectrum of inhibition substance purified from areca catechu to HPLC, optical absorptionat 280nm was resulted by aromatic ring such as tyrosin, tryptophane, and phynylalanine. For that reason, ninhydrin reaction was performed with that inhibition substance. The color is changed to purple if amino acid is present in ninhydrin reaction, but there was no change in color and was negative reaction. If 2.5% of $FeCl_3$ is applied to phenolic compound containing tannin and flavonoid, its color will be changed to green, purple, blue or black, but this compound changed its color to dark green and formed precipitates of dark green color at the high concentration. It was able to confirm that the inhibition substance is a phenolic compound. If compound is triterpenoid or saponin, color will be changed to reddish brown by adding sulfuric acid after acetic anhydride is added, but the result shows no changes in color. In addition, there is Mollish reaction to confirm the presence of saccharide in sample. If 2- 3 drops of 5% α-naphthol solution in ethanol are added and sulfuric acid is added next, reddish purple ring can be observed. Therefore, sample contains saccharide. By putting those various results together, it is able to estimate that elastase inhibition substance from areca catechu is a phenolic compound containing sugar.

Generally, we consider bonatical element containing aromatic ring substituted by one or more OH group as a phenolic compound, and these phenolic compounds exist as glycoside combining with sugar in many cases so they are water-soluble. Phenolic compound is known to be antibiotics, so many researchers report that it has protective action from mycosis, bacteria, and virus (Biochemistry of plant phenolic, Plenum, N.Y., pp557-588, 1977). In addition, many other effects such as anti-cancer effect, hypotensive,

contraceptive operation, liver protection process, and spasmolytic are discovered. By this invention, phenolic compound purified from areca catechu, which is very effective in elastase inhibition, hyaluronase, inhibition, tyrosinase inhibition, and free radical elimination, is provided, and it is possible to prepare functional cosmetics containing substance that has anti-skin aging solution.

[Experimental Example 5]

[Test on Human fibroblast proliferation effects]

1) Test method

Human normal fibroblast was inoculated into well of 96-well microplate to form $1x10^4$ cell and cultivated for 24 hours at DMEM. After cultivation, phenolic compound of preparation example 1 was dissolved into dimethyl sulfoxide and diluted with buffer solution. After changing to DMEM medium which contains no serum and its concentration is adjusted to 250㎕/㎖, it is cultivated for 24 hours more. MIT solution[3-(4,5-dimethyl-thiazole-2-yl) 2.5-diphenyl tetrazolium bromide: 5㎎/㎖] 10㎕ each was added and it was kept for 4 hours. 100㎕ of dimethyl sulfoxide solution was added to each well and stirred for 20 min and optical absorption was measured using microplate reader. Table 5 is the record and following method is to calculate cell proliferation.

$$\text{Cell multiplication Percentage(\%)} = \frac{(\text{Optical absorption of what is treated with extract} - \text{Optical absorption of comparison})}{\text{Optical absorption of comparison}} \times 100$$

2) Test results

[Table 5] Cell proliferation at Human fibroblast

Extract	Cell proliferation. effect (%)
Areca catechu L.	28.7
Phenol compound Separately urified	48.2

We can see that the areca extract and purified phenolic compound is very effective in wrinkle elimination, proliferation of fibroblast, and increasing of skin elasticity. The proliferation of fibroblast is closely related to biosynthesis of collagen, elastin, integrin, and laminin.

[Experimental Examples. 6]

[Clinical test on skin wrinkles]

Clinical test on skin's wrinkles was taken over its cosmetics prepared by example and comparison example.

20 women of 30 yrs (average 37.7) old were divided into 2 groups. Example was applied to the eyes of the women of group A, and comparison example was applied to group B for 12 weeks. Objective evaluations of expert and subjective evaluations of testant were classified into 6 levels to measure improvements (w) in wrinkle elimination. Results are as follow;

Evaluation standard for wrinkle elimination:

-3: worst

-2: worse

-1: little worse

0: no changes

1: little improved

2: improved

3: greatly improved

[Table6] Clinical test for wrinkle elimination (Expert's objective evaluation)

	Improvements (□ w) in wrinkle elimination			
	Example		Comparison example	
	T0	T12	T0	T12
Testant 1	0	3	0	1
Testant 2	0	3	0	1
Testant 3	0	3	0	1
Testant 4	0	2	0	1
Testant 5	0	2	0	2
Testant 6	0	3	0	1
Testant 7	0	2	0	1
Testant 8	0	3	0	2
Testant 9	0	2	0	1
Testant 10	0	2	0	1
□ w (T-TO)		2.5		1.2

[Table7] Clinical test for wrinkle elimination (Testant's subjective evaluation)

	Improvements (□ w) in wrinkle elimination			
	Example		Comparison example	
	T0	T12	T0	T12
Testant 1	0	2	0	1
Testant 2	0	3	0	1
Testant 3	0	3	0	1
Testant 4	0	3	0	1
Testant 5	0	3	0	2
Testant 6	0	2	0	1
Testant 7	0	1	0	1
Testant 8	0	3	0	2
Testant 9	0	3	0	1
Testant 10	0	1	0	1
□ w (T-TO)		2.4		1.2

We can notice that objective and subjective evaluation are 2.5 and 2.4 each showing that wrinkle elimination effect is very high in the example of the present invention. It is clear that the result from example is increased 100% in both subjective and objective evaluations, and it refers that improvement of wrinkle elimination has achieved.

[Experimental Examples. 7]

[Clinical test for skin elasticity improvements]

Clinical test on skin's elasticity was taken over its cosmetics prepared by example and comparison example.

At the temperature of 24~26^{0}C, humidity of 75%, to the subject of healthy 15 women (average 36.3 yrs old), example was applied to left side of face, and comparison example to right side of face for 12 weeks. And elasticity was measured using Cutometer(SEM 575, C+K Electronic Co., Germany). Results are as following table 8. Every record is written in □ R8 of Cutometer SEM 575 and □ R8 means viscoelasticity of skin.

[Table8] Clinical test for skin elasticity

	Elasticity
Operation example	0.31
Comparison example	0.15

As shown in table 8, skin elasticity of example has increased 106% from the comparison example. The phenolic compound purified from areca

extract accelerates effectively protein synthesis such as collagen or elastin through promultiplication of fibroblast and decomposition of elastin or collagen by inhibition of elastase so that skin elasticity can be rapidly improved.

[Test 8]

[Clinical test on pigmentation reversion (whitening effects)]

1) Test method

For clinical test on pigmentation reversion of cosmetic composition, 20 men and women who have freckles, lentigines, and pigmentation were divided into 2 groups. Example is applied to the testants' skin of group A and comparison example is applied to the testants' skin of group B, 2 times per day, for 12 weeks. Changes on skin color light (□ L) were measured using Chromameter Minolta CR300. Objective and subjective observations taken in the naked eyes by experts and testants respectively, were classified by levels. Table 9 shows the results.

Evaluation standard for pigmentation reversion:

-3: worst

-2: worse

-1: little worse

0: no changes

1: little improved

2: improved

3: greatly improved

[Table9] Clinical test for pigmentation reversion

Testant	Changes on luminosity		Objective evaluation By experts		Subjective evaluation By testant	
	A	B	A	B	A	B
1	5.0	4.6	3	2	3	3
2	5.2	3.0	3	1	3	2
3	6.1	5.1	2	2	2	1
4	4.9	2.2	2	1	2	1
5	5.6	3.2	3	2	2	1
6	6.2	3.1	2	2	3	2
7	3.9	2.5	2	1	2	1
8	6.6	4.3	2	1	3	1
9	6.4	4.0	3	1	1	1
10	5.5	3.9	1	1	3	2
AVR	5.54	3.59	2.3	1.4	2.4	1.5

As shown in table 9, average □ L value of group A is 5.54 and it is 54% higher than comparison example that is 3.59. Therefore, it is clear that this composition is very effective in pigmentation reversion.

Evaluations by experts show the same result as those by testants.

With these results in hand, the present invention provides cosmetic composition containing phenolic compound separated and purified from areca catechul L. This cosmetic compound has no limits in its form of products. For example, skin lotion, milk lotion, nutrition cream, massage cream, pack, moisturizing facial lotion, cleansing cream, cleansing water, essence, or powder are available for cosmetic compound products.

Form 1. Skin lotion

Compound element	Weight (%)
Phenolic compound (production ex.)	0.005
Arbutin	1.0
1.3 Butylene glycol	6.0
Glycerin	4.0
Oleic alcohol	0.1
Polyoxyethylene Sorbitan Monolaurate	0.5
Ethanol	15.0
Benzophenones	0.05
Aroma, antiseptic	0.3
Purified water	To 100

Form 2. Nutrition Lotion (Milk lotion)

Compound element	Weight (%)
Phenolic compound (production ex.)	0.001
Arbutin	2.0
Propylene glycol	6.0
Glycerin	4.0
Triethanolamine	1.2
Tocopheryl acetate	3.0
Liquid paraffin	5.0
Squalene	3.0
Macadamia Nut oil	2.0
Polyoxyethylene Sorbitan Monostearate	1.5
Sorbitan sesquioleate	1.0
Carboxy vinyl polymer	1.0
BHTE	0.01
EDTA-2NA	0.01
Aroma, Antiseptic	0.3
Purified water	To 100

5

Form3. Nutrition Cream

Compound element	Weight (%)
Phenolic compound (production ex.)	0.001
Arbution	2.0
Cetosthearyl alcohol	2.0
Glyceryl stearate	1.5
Trioctanoin	5.0
Polyoxyethylene Sorbitan Monostearate	1.2
Sorbitan stearate	0.5
Squalene	5.0
Liquid paraffin	3.0
Cyclomechicon	3.0
BHTE	0.05
Delta-tocopherol	0.2
Glycerine	4.0
1.3- Butylene glycol	2.0
Xanthan gum	0.1
EDTA-2NA	0.25
Aroma, Antiseptic	0.3
Purified water	To 100

Form 4. Massage Cream

Compound element	Weight (%)
Phenolic compound (production ex.)	0.001
Arbutin	2.0
Propylene glycol	6.0
Glycerin	4.0
Triethanolamine	0.5
Bees wax	2.0
Tocopheryl acetate	0.1
Polyoxyethylene Sorbitan Monostearate	3.0
Sorbitan sesquioleate	2.5

Stearyl alcohol	2.0
Liquid paraffin	30.0
Carboxy vinyl polymer	0.5
Aroma, Antiseptic	0.3
Purified water	To 100

Form 5. Pack

Compound element	Weight (%)
Phenolic compound (production ex.)	0.001
Arbutin	2.0
Propylene glycol	2.0
Glycerin	4.0
Carboxy vinyl polymer	0.3
Ethanol	7.0
PEG-40 hydrogebitide caster oil	0.8
Triethanolamine	0.3
BHTE	0.01
EDTA-2NA	0.01
Aroma, Antiseptic	0.3
Purified water	To 100

The phenolic compound extracted and purified from areca catechu has very excellent inhibition activity on porcine pancreas elastase (PPE) and human neutrophil elastase (HNE), and this phenolic compound shows competitive reaction with substrate to PPE and HNE. Furthermore, this phenolic compound has an ability to eliminate free radical and the free radical eliminating effect is much higher than that of standard compound. Inhibition process on hyaluronidase, which is activated in mast cell, is very effective. In addition, inhibition activity for tynosinase, known as whitening effect, is also great.

CLAIMS

1. An anti-aging cosmetic composition which has both of whitening and wrinkle elimination effects, comprising phenolic compound extracted from areca catechu L.

2. The cosmetic composition claimed in claim 1, wherein 0.00001~5.0 weight % of phenolic compound is comprised.

3. The cosmetic composition claimed in claim 2, wherein 0.001~1.0 weight % of phenolic compound is comprised.

4. The cosmetic composition claimed in claims 1, 2 or 3, wherein the phenolic compound can be prepared by steps:

preparing extract from seed of Areca Catechu L. with anhydrous or aqueous alcohol, or one or more organic solvent selected from the group comprised of acetone, ethyl acetate, diethyl ether, benzene, chloroform, hexane and butanol; and

obtaining phenolic compound by silica gel column chromatography, preparative TLC, and reverse HPLC from said extract.

5. The cosmetic composition claimed in claims 1 or 2, has product form selected from the group comprised of skin lotion, nutrition lotion, nutrition cream, massage cream, or pack.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00997

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 7/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC7:A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
JICST-EPLUS(STN), KOSMET(STN), CABA(STN), CAPLUS(STN), SCISEARCH(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEE K K; CHOI J D. "The effects of Areca catechu L extract on anti-aging", International Journal of Cosmetic Science, UK, 1999, Vol.21, No.4, pp. 285-295	1-5
Y	LEE K K; CHOI J D. " Areca catechu L. extract. I. Effects on elastase and aging", Journal of cosmetic science, USA, 1998,Vol.49, No.5, pp.285-297	1-5
Y	OHSUGI, M.; FAN WENZHE; HASE, K..; XIONG QUANBO; TEZUKA, Y.; KOMATSU, K.; NAMBA, T.; SAITOH, T.; TAZAWA, K.; KADOTA, S.; FAN, W. Z.; XIONG, Q. B. "Active-oxygen scavenging activity of traditional nourishing-tonic herbal medicines and active constituents of Rhodiola scara", Journal of ethnopharmacology, Japan, 1999, Vol.67, No.1, pp.111-119	1-5
Y	KUN-KOOK L; KWANG-SIK L; JEONG-HA K; BYUNG-KEE J O; JUNG-DO C. " Efficacy and biological activities of a new anti-aging agent obtained from Areca catechu" IFSCC CONGRESS : Science and beauty at the dawn of the third millenium, 14-18 september 1998, paper P018 pp.1-7	1-5
Y	KIM, B. J.; KIM, J. H.; KIM, H. P.; HEO, M. Y."Biological screening of 100 plant extracts for cosmetic use (II): anti-oxidative activity and free radical scavenging activity", International Journal of cosmetic science., UK, 1997, Vol.19, No..6, pp. 299-307	1-5

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 AUGUST 2002 (14.08.2002)	14 AUGUST 2002 (14.08.2002)

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	CHANG, Jin Ah Telephone No. 82-42-481-5602

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00997

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEE K K; DOCHOI J. " Areca catechu L. extract. II. Effects on inflammation and melanogenesis", Journal of cosmetic science, USA, 1998, Vol.49, No.6, pp.351-359	1-5
Y	LEE K K; CHOI J D. " The effects of Areca catechu L. extract on anti-inflammation and anti-melanogenesis" International Journal of cosmetic science, UK, 1999, Vol.21, No.4, pp.275-284	1-5
Y	JP5-320037 A2 (NANBA TSUNEO, MIKIMOTO PHARNACEUT CO. LTD.,) 3 DEC 1993, see the claim 1	1-5
Y	JP5-331041 A2 (NANBA TSUNEO, MIKIMOTO PHARNACEUT CO. LTD.,) 14 DEC 1993, see the claim 1	1-5
Y	KR 99-58669 A (COREANA COSMETICS CO. LTD.,) 15 JUL 1999 see the whole document	1-5
A	PADJAMA, P. N.; BAIRY, K. L.; KULKARNI, D. R. "Pro-healing effect of betel nut and its polyphenols", Fitoterapia, 1994, Vol. 65, No. 4, pp, 298-300	1-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/00997

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 5-320037 A2	03.12.1993	None	
JP 5-331041 A2	14.12.1993	None	
KR 99-58689 A	15.07.1999	None	